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14. ABSTRACT

The report herein describes progress made in the first year of EMERGE: Engineered Materials the Create Environments for ReGeneration via Electric Fields. The objective of the project is to develop a next-generation ocular implant material with biomimetic chemistry and nanotopography with unique drug delivery functionality to 1) provide appropriate biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation and 2) deliver wound electric field enhancing pharmaceuticals for enhanced bioelectronics cues. To this end, we are developing a collagen material with tailored nanotopography with ability to deliver endogenous wound electric field-enhancing molecules that will encourage and accelerate the wound healing process. We monitor wound electrophysiology as a function of time using a vibrating probe.

15. SUBJECT TERMS
Corneal, Wound healing, Collagen, Aminophylline, Nanoparticles, Drug delivery

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1. Introduction

The *objective* of the project discussed here is to develop a next-generation ocular implant material with biomimetic chemistry and nanotopography with unique drug delivery functionality to 1) provide appropriate biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation and 2) deliver wound electric field (EF) enhancing pharmaceuticals for enhanced bioelectronics cues. To this end, we are developing a collagen material with tailored nanotopography possessing the ability to deliver endogenous wound electric field-enhancing molecules that will encourage and accelerate the wound healing process. The material will be engineered to closely mimic the *in vivo* cornea environment, exhibiting excellent transparency and mechanical strength properties important for high performance cornea implants. Incorporation of drug delivery functionality both directly into the collagen membrane, and in embedded nanoparticles will allow tailored, sustained delivery of bioelectric modulators that have been shown by our team to stimulate cell responses related to the healing process. To reach our goal, our research is organized around four Aims. Aim 1 is to correlate the presence and dosage of the bioelectric modulators with wound healing to determine formula for maximizing wound EF. Aim 2 is to develop the collagen material and correlate the relationship between collagen fiber topography and wound healing to enable optimization of the material structure. Aim 3 is to incorporate the bioelectric modulators into the collagen membrane and characterize the release. Aim 4 is to evaluate and optimize the therapeutic benefit of collagen materials containing drug-loaded nanoparticles on corneal healing. The technology developed in this project will be applicable for the treatment of multiple types of injuries to ocular structures and could be extended to introduce additional pharmaceuticals, such as antibiotics and analgesics to target different aspects of ocular injury. An implantable EMERGE biocomposite would facilitate accelerated wound healing, ensure a faster return to full function, and improve the quality of life for those inflicted with eye injuries.

2. Keywords

Wound healing
Cornea
Bioelectric
Collagen
Drug delivery
Vibrating probe
Endogenous electric field
Nanoparticles
Poly (lactic co-glycolic acid) (PLGA)
Injury
Aminophylline

3. Accomplishments

Major goals and objectives

The overall objective of EMERGE is to develop an ocular implant material with biomimetic chemistry, nanotopography, and unique drug delivery functionality to improve corneal wound healing through two strategies:

1. Providing biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation
2. Delivering chemical bioelectric modulators for enhanced wound electric field

To realize this objective, the project is based around four Aims, each with an associated Task, as described in the Statement of Work (SOW). These Tasks are as follows:

1. Correlate the presence and dosage of the bioelectric modulators with wound healing.
2. Develop a collagen-collagen biocomposite material with tailorable nanostructure and collagen fibril alignment, and correlation of the relationship between collagen fiber topography and wound healing to enable optimization of the biocomposite structure.
3. Incorporate bioelectric modulators into a pharmacological delivery system within the collagen-collagen composite and characterize release.
4. Measure endogenous electric field (EF) at corneal wound sites and correlate with effects of bioelectric modulators, pharmacological delivery system, and collagen biocomposite to assess efficacy of these materials and pharmaceuticals for healing.

Each Task has smaller subtasks (specific aims), also called out in the SOW. Goals in year 1 have focused on these subtasks, ensuring progress towards achieving our overall project objective.

Accomplishments

Accomplishments are discussed below, organized by Aim. Specific Aims relevant to work during the first year of the project are identified.

Aim 1

Specific Aim 1a: Measure wound electric field (EF) and ion fluxes at corneal wounds with a vibrating probe and ion selective electrodes.

We have characterized the spatiotemporal dynamics of rat cornea wound electric currents and ion flux using the vibrating probe. Previous work focused on superficial epithelial wounds of ~30 μm deep. In EMERGE, we have focused on measurements of deeper wounds of approximately 100 μm in depth, penetrating the

stroma. For both shallow and deep wounds, electric current and ion flux are maximized at the wound edge, smaller at the wound center, and reduced to normal (unwounded) values a small distance outside the wound. Wound electric current is always outward (outward flow of positive current). In previous studies of shallow wounds, we have observed a transient outward flux of potassium, which diminished rapidly and therefore appeared to be leakage. We have also observed small fluxes of calcium and sodium. The main ion flux appeared to be a large influx of chloride ions at the wound edge. We expect ion flux observations in deeper wounds to be similar and plan to measure ion flux in deeper wounds with ion selective probes in the near future. Timelapse measurements of electric current in the hours after wounding indicate that the wound electric signal is long-lasting and appears to be actively-regulated. The wound electric signal increases rapidly until approximately 40 minutes after wounding and is maintained at a high level for several hours. **Figure 1** shows time lapse data for both a shallow (normal epithelial) wound and a deep (stromal) wound. In both cases, the electric signal is significantly larger than the normal unwounded signal for at least eight hours after wounding.

Previously, we made epithelial wounds manually using a biopsy punch. To replicate a more traumatic cornea wound that penetrates both the epithelium and stroma, we are currently using a Nidek EC-5000 excimer laser. We optimized the laser settings to obtain circular wounds 2 mm in diameter and approximately 100 μm deep. We confirmed wound depth using optical coherence tomography (OCT). Initially, corneas with laser wounds (2 mm x 100 μm) showed evidence of cell damage in the epithelium outside the wound, possibly due to heat damage from the laser. We optimized the laser application by first chilling eyes in a saline ice bath prior to wounding, and then applying the laser in 10 second bursts, with 5 second rest in between to minimize laser damage outside the wound. The wound edge electric signal measured from optimized stroma cornea was not significantly different from that of the shallower epithelial wound (normal: 4.19 vs laser: 3.96 $\mu A/cm^2$; $P>0.7$). The dynamic time course of the electric signal change in the hours after wounding was also similar to that of shallower wounds (**Figure 1**). Normal epithelial wounds 2 mm in diameter heal almost completely in 48 h. Deep laser wounds took longer to heal than shallow epithelial wounds, but did heal 100% by day 8 (**Figure 2**).

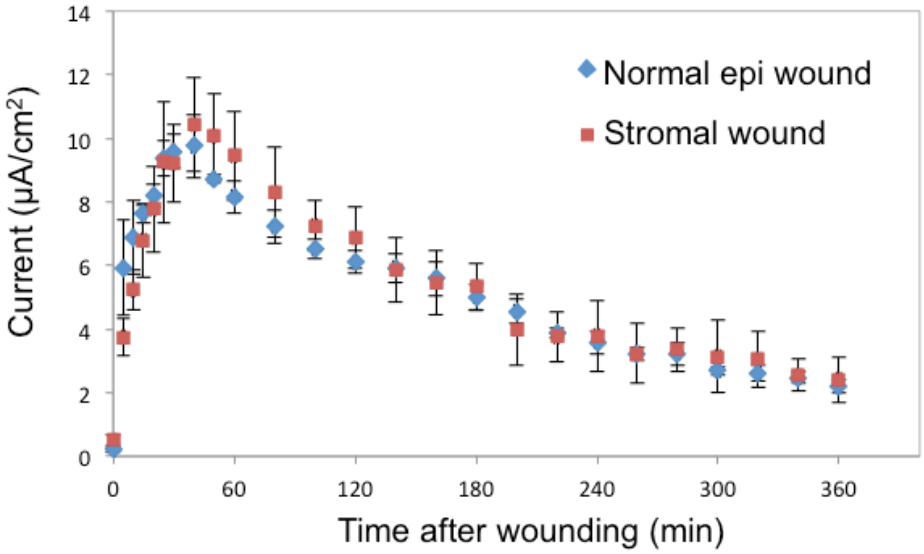


Figure 1. Comparison of electric signal at normal shallow epithelial wound (~30 μm deep) with deep stromal wound (~100 μm). Values at time zero show small current present at the intact cornea before wounding. Electric current was measured with a vibrating probe. Measurements were taken at the wound edge.

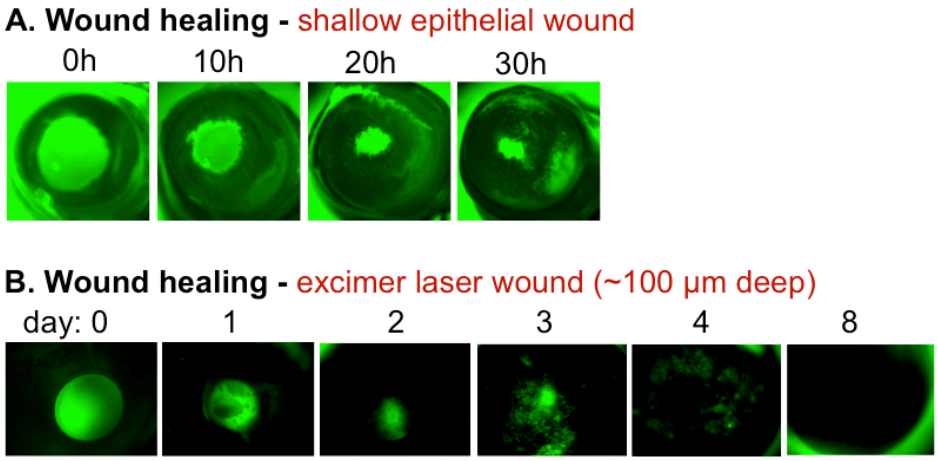


Figure 2. Wound healing. A. Shallow epithelial typically heals within 48 h. (Previous work, shown for sake of comparison.) B. Deeper stromal laser take longer to heal, and were fully healed by day 8. Wounds were visualized with fluorescein dye. The wound is the bright area in the center. Scale bar 1 mm.

Specific Aim 1b: Quantify effects of pharmacological agents on corneal wounds and resulting EF using rat corneal model.

To guide the choice of bioelectric modulators to explore in EMERGE, we revisited prior work from our laboratories. Previously, we used specific pharmacological activators (aminophylline, ascorbic acid*) or inhibitors

(furosemide) of ion flux and investigated their effects on cornea wound electric signal and wound healing (shallow epithelial wounds). Aminophylline and ascorbic acid (10 mM) both significantly increased the cornea wound electric signal ($P<0.04$, $P<0.03$, respectively). Aminophylline also significantly increased the chloride flux at the wound ($P<0.03$). Both of these drugs also significantly enhanced cornea wound healing ($P<0.01$, $P<0.05$, respectively). Wounds 3.5 mm in diameter that normally took 48 h or more to completely heal were healed in 30 h in the presence of these drugs. Furosemide significantly inhibited wound electric signal ($P<0.01$) and also slowed wound healing ($P<0.05$). *[*Aminophylline is a nonspecific phosphodiesterase inhibitor that increases cAMP levels and enhances Cl^- flux. Ascorbic acid increases Na^+ and Cl^- transport across amphibian cornea. Furosemide inhibits the $Na^+/K^+/Cl^-$ cotransport system.]*

Based on the information above, for initial measurements in EMERGE, we have chosen to focus on the use of aminophylline as a bioelectric modulator to enhance wound healing. To ensure that the aminophylline has the same effect on deep stromal wounds as shallow epithelial wounds, we measured wound electric field using the vibrating probe with and without the presence of 10 mM aminophylline. The presence of aminophylline appears to result in an increase in the wound electric field. (**Figure 3**) Although data shown below are not statistically significant, we are in the process of collecting more measurements to improve statistics.

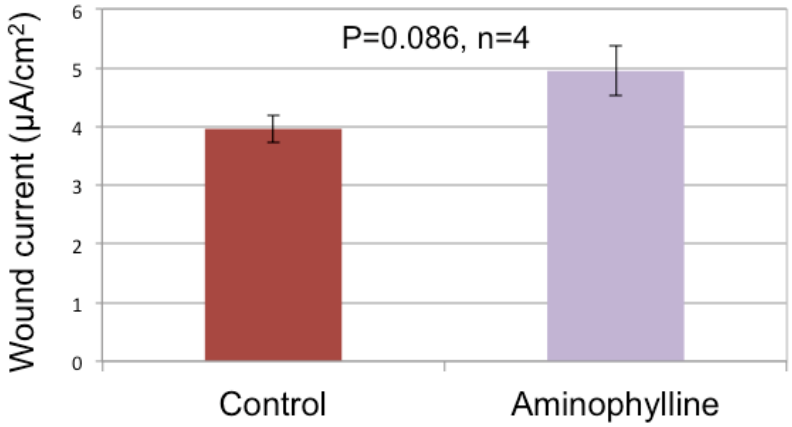


Figure 3. In a rat cornea model, aminophylline (10 mM) increased cornea wound electric signal ($P<0.01$).

Specific Aim 1c: Using the human cornea test platform, determine optimal concentrations & combinations of pharmacological agents for regulating wound electric signals to maximally stimulate the endogenous EF and thus facilitate wound healing and regeneration of injured cornea.

Because the rat model shows a similar dynamic time course of rising and maintained electric current as a human cadaver eye, the rat model has been the focus of work on this aim to date. As seen i

n Figure 3, the presence of 10 mM aminophylline appears to increase wound electric field, which we expect to facilitate wound healing and regeneration of injured cornea. Further investigation is ongoing.

Future work will focus on further optimization of aminophylline concentration and dosage. We will also further characterize wound electrophysiology of deep stromal wounds with ion selective probes.

We have made significant progress toward identification of pharamacological agents and their specific dosages and combination desired for maximization of endogenous EF, the *Milestone* associated with *Specific Aim 1*.

Aim 2

Specific Aim 2a: Synthesize collagen-collagen composites consisting of electric-field aligned collagen vitrigels and electrospun collagen nanofibers.

We have synthesized engineered collagen membranes in the presence of electric fields. Synthesis parameters can be tuned to achieve collagen materials with varying fiber diameters, fiber density, thickness-normalized transparency and suture strength. Mold parameters can be changed to tailor collagen membrane thickness and areal dimensions. The protocol for collagen membrane synthesis is as follows: Collagen solution of a known concentration is placed in a metallic mold. The metallic mold is placed between, but not in contact with, two metallic parallel plates. In some cases, there is a potential between to the two plates, subjecting the collagen to a static electric field for a certain duration of time (on the order of hours). Subsequently, a gelation process is performed using a buffer solution. Following gelation, single layers of the collagen membrane are removed from the mold and combined into multi-layer constructs. Finally, a dehydrothermal cross-linking technique is used to increase cross-link density.

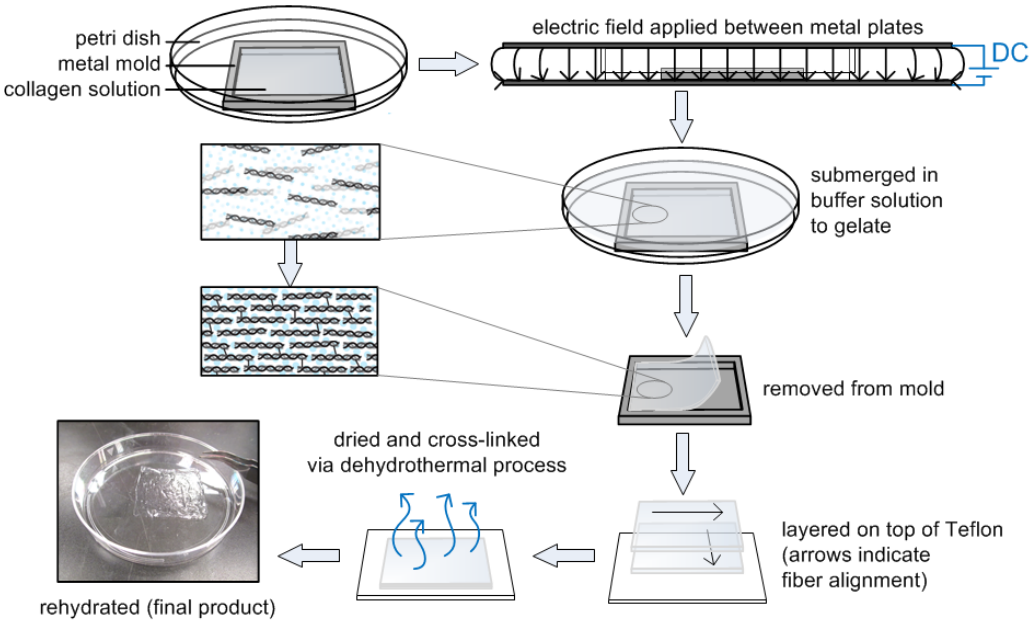


Figure 4: Schematic depicting collagen membranes formed in the presence of an electric field.

Specific Aim 2b: Characterize strength and transparency of collagen-collagen composites and correlate with synthesis parameters and material structure.

Techniques used to fabricate collagen membranes are highly tunable, which can result in materials with widely varying structures and properties. As such, a Design of Experiments (DOE) was conducted in an effort to correlate the membrane synthesis parameters with resulting structure and properties. A DOE is a statistically-based method of conducting a series of experimental runs in which independent variables (often called factors) are simultaneously varied while obtaining a desired effect (often called responses). It has been shown that this method of designing experiments is much more efficient than changing a single variable at a time. The DOE method has been used in many applications and excels at elucidating detailed relationships when there are many factors and many levels for each factor.

For the DOE employed herein, factors included collagen concentration (5, 10 mg/mL), dialysis (yes, no), electric field (5, 10 V/cm), electric field exposure time (4, 8 hours), processing temperature (4, 23, and 37°C), and collagen solution pH (1,2.5 and 4.) The design called for 12 runs. Factors for each run are shown in Table I. Responses measured for samples in this DOE include transmittance, suture strength and microstructural details (fiber width and degree of alignment). The results for transmittance of a sample at 550 nm, normalized to a thickness of 150 μm are shown in Table I as well. The goal of the DOE is to produce factor-structure-properties relationships for the collagen membranes, such that we can rationally tailor the materials to facilitate optimized performance.

Run	Collagen Concentration (mg/mL)	Dialysis	Electric Field (V/cm)	Electric Field Time (Hours)	Processing Temperature (˚C)	pH	Normalized Transmittance at 550 nm (%) (150 µm)	Suture Strength Normalized Max Load x 10^{-4}(N/µm)
1	10	No	10	8	37	2.5	83 ± 4	
2	10	Yes	5	8	37	4	92 ± 4	4 ± 3
3	5	Yes	5	8	4	2.5	97 ± 5	2 ± 1
4	5	Yes	5	4	37	1	73 ± 6	7 ± 2
5	10	No	5	4	4	4	92 ± 8	1.1 ± 0.3
6	10	Yes	10	4	4	2.5	96 ± 9	0.9 ± 0.2
7	10	No	5	8	23	1	76 ± 6	2 ± 1
8	10	Yes	10	4	23	1	72 ± 5	7 ± 4
9	5	No	5	4	23	2.5	87 ± 4	3 ± 2
10	5	No	10	4	37	4	78 ± 12	6 ± 3
11	5	Yes	10	8	23	4	90 ± 3	4 ± 1
12	5	No	10	8	4	1	64 ± 4	3 ± 1

Table 1: Factors defining the 12 DOE runs, and results for transparency and suture strength characterization.

Transmittance was measured with a Perkin Elmer Lambda 950 UV-Vis Spectrometer. At least 9 measurements were taken for each fully hydrated collagen sample and normalized for a thickness of 150 µm, average transmittance values at 550 nm range from 64 to 97%. The transmittance values reported here exceed those of collagen vitrigel (CV) materials previously made at APL (60%).

Suture strength measurements were made following a protocol derived from Ref.[1]. Briefly, fully hydrated collagen membranes are cut into 8 mm discs. Two standard 10-0 sutures (33 um monofilaments) were pushed through the collagen discs 2 mm inside opposite edges. Sutures were placed in pneumatic grips of an Instron 5942 with a 500 N load cell. In the setup, collagen membranes are suspended between the two sutures. The sutures were drawn until break and force at break was recorded. Average force at break derived from suture tests range from 0.9 to 7 x 10^{-4} N/µm. The high suture strengths exceed previously published data for collagen membranes.[11] High suture strength ensures that surgeons will be able to easily manipulate the material and successfully suture the material into a wound. We have previously received positive feedback from surgeons following *in vivo* studies with similar materials.

Initial interpretation of the DOE indicates that sample transmittance depends primarily on the two factor relationship of dialysis*processing temperature as well as collagen concentration. Higher transmittance values are obtained when dialysis is performed (removing salts) and processing temperature is high. Further analysis of DOE results, including structural characterization (discussed below) will allow elucidation of the mechanism allowing for higher transmittance data.

Suture strength is highly dependent on processing temperature, with a higher temperature correlating with a high force at break.

[1] 1. Li et al. "Recruitment of multiple cell lines by collagen-synthetic copolymer matrices in corneal regeneration," Biomaterials (2004)

Structural characterization of the membranes is ongoing. We are collecting transmission electron microscopy images, as well as scanning electron microscopy images for various collagen gels. Example transmission electron microscopy images of one of the collagen membranes fabricated in this study is shown below in **Figure 5**.

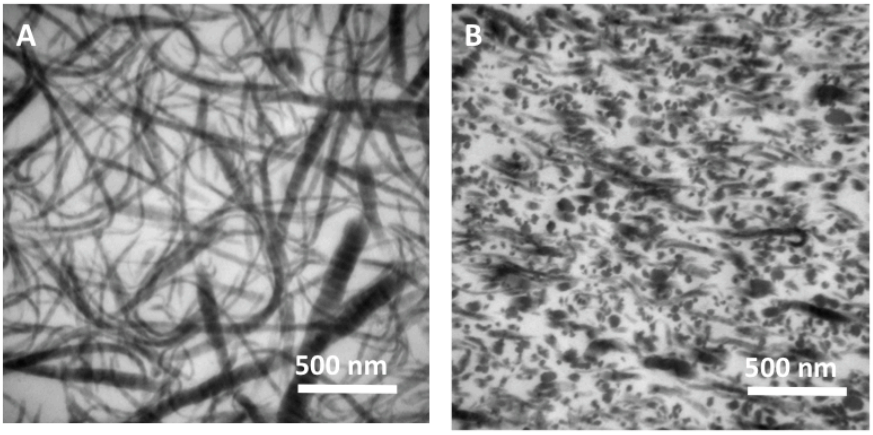


Figure 5: Transmission Electron Micrograph of collagen gel. A. Top view. B. Side view. Note the banded collagen structure visible in A.

In the near future, we plan to perform a primary down-selection of materials that have average transmittance of exceeding 75% (to maintain vision) and a sufficient suture strength (corresponding to a break point of at least 0.2 mN/μm). We will then perform a second down-selection of at least 3 materials that meet these requirements but have very different morphologies. We will work with these 3 materials for the remainder of the project.

Specific Aim 2d: Use the vibrating probe and ion selective electrodes to map the electrical signal at the cornea wounds (rat model) and variations resulting from application of the collagen composite (with varying topography) to the injured area. Particular attention will be focused on characterizing the collagen materials specific intrinsic properties.

Following fabrication, collagen membranes are dehydrated. They are then rehydrated with a saline solution prior to *in vitro* experiments. To characterize the rehydration dynamics, we collected optical coherence tomography (OCT) images throughout the rehydration process. Collagen membranes appeared to reach maximum thickness after 24 h in saline. No further increase of thickness was seen if the gel was rehydrated for more than 24 h.

We next characterized the effects of two distinct rehydrated collagen membranes (with a nominal thickness of 100 μm) on cornea wound electric signal, electric field enhanced collagen (EF), and non electric field enhanced collagen (No EF). With the collagen (a 2 mm circular plug made with a biopsy punch) in place the wound edge electric signal was slightly less than normal, but not significantly lower (normalized to percentage: normal 100%, EF 93.7%, No EF 89.8%; P>0.4) (**Figure**

6). We showed that the distance the vibrating probe is from the sample being measured affects measured values. As the probe is moved away from the source of electric signal, the current detected falls exponentially. We therefore tested the effect of physical presence of collagen membrane and probe distance from wound edge on the electric signal. We placed the probe in measuring position as close as possible to the wound edge with the collagen membrane in place and recorded the electric signal. We then removed the collagen membrane and the electric signal increased only slightly (EF 11%, No EF 14%). We then moved the probe close to the wound edge and the measured signal increased dramatically (EF 115%, NA 96%). The physical presence of the collagen membrane therefore has minimal effects on the electric signal.

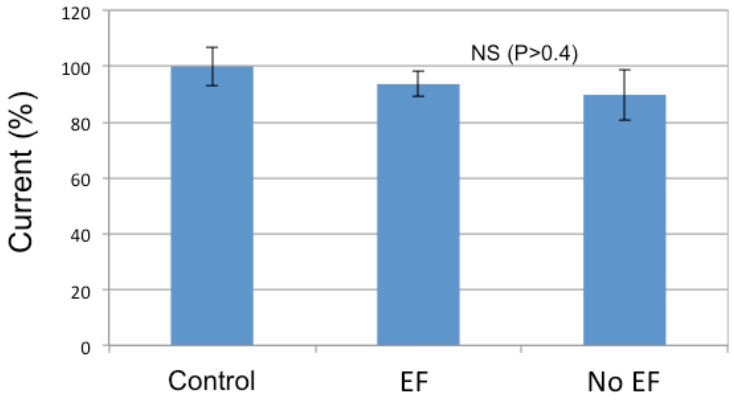


Figure 6. The presence of collagen (100 µm thick) at the wound caused a small, but insignificant, reduction of measurable electric signal. Electric field enhanced collagen (EF) and non-electric field enhanced (NEF) had similar effects. In this case, the Control is a stromal wound with no collagen present..

For long-term collagen membrane attachment during wound healing assays, we tested fibrin adhesives (Evicel and Tisseel). The best method appeared to be placing the collagen (2 mm x 100 µm thick) onto the laser wound (2 mm x 100 µm deep) and placing a drop of the fibrin adhesive over the cornea to hold the collagen in place. Using this method, the collagen membrane was held in place for duration of the experiment (4+ hours.) See **Figure 7**.

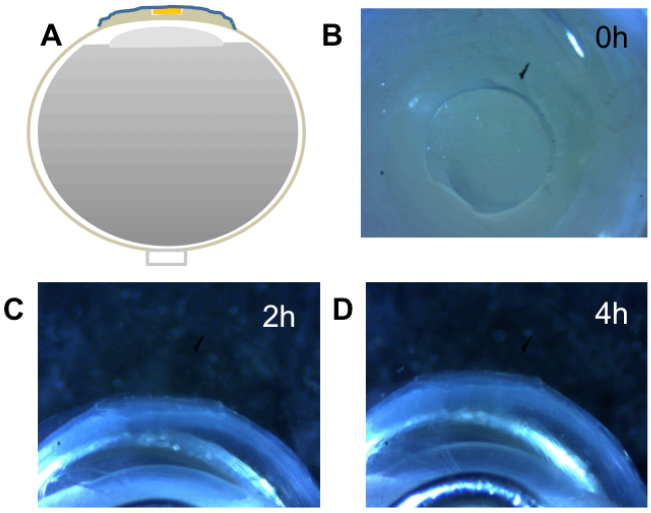


Figure 7. A. Side view schematic of eye with collagen membrane (orange) held in place by fibrin adhesive (blue). **B.** Top view of laser wound with collagen membrane held in place by fibrin adhesive. **C,D.** Side views of collagen membrane at 2 h and 4 h after application.

Future work will focus on completing remaining characterization of collagen membrane materials and selecting at least three membrane materials to use in wound healing studies. Using the vibrating probe technique, we will correlate the effect of collagen topography on endogenous EF (wound electrophysiology) and wound healing.

Aim 3

Specific Aim 3a: Synthesize nanocarriers for encapsulation and delivery of bioelectric modulators, as required based on Tasks 1b & c; and Specific Aim 3b: Encapsulate bioelectronics modulators in nanoparticles and characterize modulator loading, release kinetics and drug stability.

We have chosen to focus on optimization of the release of aminophylline, a bioelectric modulator with well-demonstrated effect on endogenous electric field and wound healing, identified previously. We first characterized the release of aminophylline directly from the collagen membrane material. For these experiments, dehydrated collagen membranes were rehydrated in a solution with a known concentration of aminophylline. Release will be subsequently characterized by monitoring the optical absorption of a charge transfer complex between aminophylline and sodium 1,2-naphthoquinone-4-sulphonate (NQS), providing a UV absorption-based method for the determination of aminophylline concentration. Characterization is ongoing.

Poly(lactic-co-glycolic) acid (PLGA) nanoparticles have been selected to encapsulate and deliver aminophylline. PLGA is extremely biocompatible and there

is precedent for PLGA use in FDA approved therapeutics. When aminophylline is encapsulated in a PLGA shell, gradual dissolution of the shell will result in the slow release of aminophylline to the wound site. Release profiles can be tailored by varying nanoparticle loading into the collagen membrane and nanoparticle chemistry.

The aminophylline loaded PLGA nanoparticles are made as follows. First, an aqueous buffered solution of aminophylline of known concentration is prepared. Separately, a solution of PGLA and Pluronic F68 in ethyl acetate is prepared. The aminophylline solution is transferred into the PLGA solution and the mixture is briefly sonicated. During sonication, the solution is kept over ice to avoid overheating. The resulting water in oil emulsion is placed into a second aqueous buffered bath, with Pluronic F68. The mixture is again briefly sonicated. The resulting water/oil/water emulsion is placed into a rotary evaporator to remove ethyl acetate. Following solvent removal, the solution is centrifuged. Effluent is discarded and particles are re-dispersed in deionized water. This washing procedure is carried out three times. The resulting purified nanoparticle solution is flash frozen with liquid nitrogen and lyophilized to afford a dry powder consisting of nanoparticles with a diameter of approximately 125 nm (**Figure 8**). Characterization of the aminophylline release from nanoparticles is ongoing.

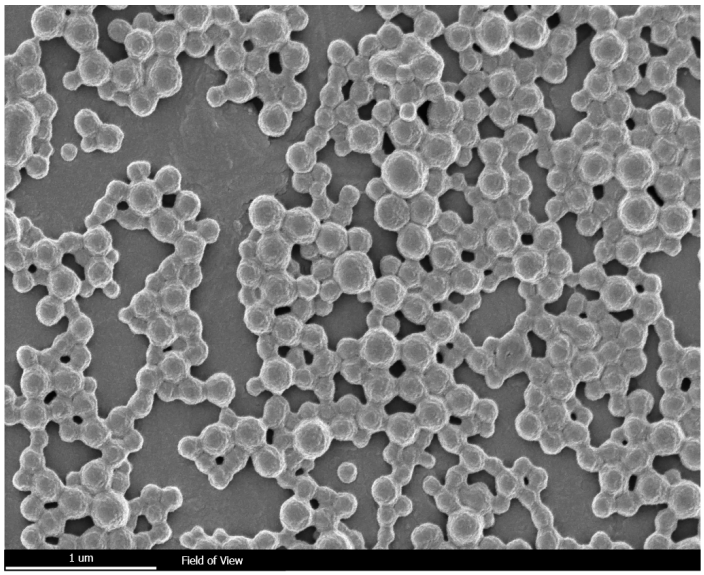


Figure 8: Scanning Electron Micrograph of PLGA nanoparticles.

Opportunities for training and professional development

Nothing to report for Year 1.

In the coming year, we plan to work with a high school intern. We also plan for some contributors of this project to attend conferences, listed in a later section.

Dissemination of Results

Nothing to report for Year 1.

We plan to publish a paper describing the aminophylline-loaded PLGA nanoparticle synthesis in year 2. We will also assemble a manuscripts on electric field enhanced collagen membranes, and wound healing of stromal wounds in year 2.

We have a goal of disseminating results at a technical conference in year 2. We will consider submitting abstracts to at least one of the following:

- The Association for Research in Vision and Opthamology, ARVO 2016, May 1-5, 2016
- The Materials Research Society Fall Meeting MRS Fall 2016, Nov. 27-December 2, 2016
- Biomedical Engineering Society Annual Meeting, BMES 2016, Oct 5-8 2016
- Biology and Pathology of the Cornea, Gordon Research Conference, Feb 27-28, 2016

Future plans to Accomplish Goals and Objectives

For the next reporting period, we will focus on the following:

- Examining wound healing of deep laser wound healing with and without pharmacological drug (10 mM aminophylline).
- Examining wound healing with collagen membranes present.
- Examining wound healing with collagen membranes present, releasing aminophylline
- Characterization of collagen membrane gel microstructure to complete DOE analysis and interpretation, and allow collagen membrane down-selection.
- Characterization of aminophylline release from collagen membrane gel and from synthesized nanocarriers, separately and as a system.

4. Impact

EMERGE is aimed at developing a next-generation ocular implant material with biomimetic chemistry and nanotopography with unique drug delivery functionality to 1) provide appropriate biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation and 2) deliver wound electric field enhancing pharmaceuticals for enhanced bioelectronics cues. We are using a unique characterization tool, the vibrating probe, to measure the endogenous electric field at the wound edge. The novel aspects of this project keep us at the forefront of biomaterials (specifically collagen-based biomaterials) and wound healing as it relates to endogenous electric fields (and their manipulation). To the best of our knowledge, we are the first to synthesize aminophylline loaded PLGA nanocarriers, and to combine these nanocarriers with a uniquely engineered collagen material. We have filed internal IP Disclosures at JHU/APL to protect IP related to the engineered collagen membranes as well as the aminophylline-loaded nanocarriers. (IP disclosures have been filed with iEdison.)

A successful project will have a considerable benefit to both the military and society at large. Despite representing only 12% of the body's surface area, the head, face and neck body region encounters 30% of combat wounds, an increase from prior conflicts. Ocular injuries, specifically traumatic eye injury from penetrating wounds and traumatic brain injury-related visual disorders, rank second only to hearing loss as the most common injury among active military. In contrast to the 80% return-to-duty rate for most battle trauma injuries, only 20% of these eye-injured warfighters return to duty.

The standard treatment for abrasions is the application of an ophthalmic antibiotic ointment and patching of the affected eye(s) for 24 hours or longer. This therefore causes an inevitable and unpredictable period of loss of full function of the soldier. Current treatments for penetrating injuries rely on use of donor amniotic membrane, which is expensive, scarce in field hospitals, difficult to work with surgically, and can lead to host rejection. The development of a biomaterial that can repair multiple layers of the cornea, treat corneal scarring, or even repair full thickness injuries would be a significant advance in restoring native tissue architecture and function. An implantable EMERGE biocomposite would accelerate wound healing, ensure a faster return to full function, and improve the quality of life for those inflicted with eye injuries. Additionally, this EMERGE biocomposite could be used as a single, point-of-care therapeutic material, with integrated pharmaceutical delivery and healing benefit, enabling first responders to effectively treat casualties on the battlefield as close to time of injury as possible.

5. Changes/problems

There have been no major changes to the Statement of Work or to our approach.

Minor details in outlined experiments have been made, listed here:

- Due to high performance of collagen membrane alone, collagen electrospun fibers do not need to be included in the collagen membranes, as originally suggested in the SOW. (Aim 2)
- Optimized nanocarriers for the bioelectric modulators have been identified as PLGA nanoparticles, an option not explicitly called out in the SOW. (Aim 3)
- We have chosen to continue studies using a rat cornea instead of a human cadaver eye. Rat cornea wounds have a larger wound electric field and they are more readily available. (Aim 1)

None of these changes are expected to change timeline, budget, or spirit of the project.

6. Products

Publications

To date, we have not published work done in EMERGE in scientific journals. We plan to publish first results from EMERGE in year 2 of the project.

Technologies and Techniques

Work in this project is contributing to the development of new technique. We have developed protocols to synthesize strong, transparent, collagen membranes using unique recipes involving exposure to electric fields. We have also developed aminophylline-loaded PLGA particles. For both of these techniques, internal IP disclosures have been filed. The IP disclosures, listed below, have been filed with iEdison.

Titles of submitted IP Disclosures:

"Aligned collagen materials with drug delivery capabilities for enhanced wound regeneration"

"Aminophylline loaded poly(lacto-co-glycolic acid) nanoparticles for ocular wound healing."

7. Participants and other collaborating organizations.

Personnel	**Role**	**Approximate Effort**
JHU/APL		
Morgana Trexler	Principal Investigator	160 hrs
Leslie Jimison	Materials Scientist, Project Manager	360 hrs
Xiomara Calderon-Colon	Materials Scientist, Collagen materials synthesis, drug delivery	600 hrs
Lance Baird	Chemist, Drug delivery	400 hrs
James Beaty	Program Manager	50 hrs
UC Davis		
Min Zhao	Co-PI	160 hours
Brian Reid	Scientist, Electrophysiology	560 hours
Fernando Ferreira	Scientist, Electrophysiology	56 hours

8. Special Reporting Requirements

Quad chart:

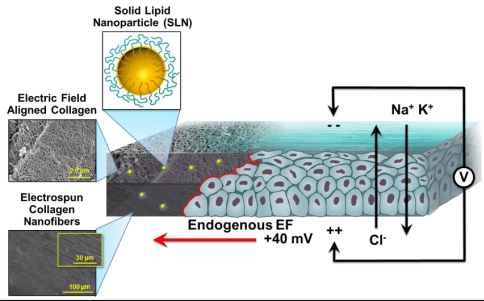
EMERGE –
Engineered Materials that create Environments for ReGeneration via Electric Field
DUNS: 00-191-0777, EIN: 52-0595110,Log Number: MR130110

PI: Morgan Trexler, Ph.D.
Co-PI: Min Zhao, M.D., Ph.D.
Org: Johns Hopkins University Applied Physics Laboratory
Org: UC Davis
Proposed Award Amount: $1M

Study Goal: To develop an ocular implant material with biomimetic chemistry and nanotopography and unique drug delivery functionality to 1) provide biochemical and biophysical cues for enhanced cell migration, differentiation and proliferation and 2) deliver wound electric field enhancing pharmaceuticals for enhanced bioelectronics cues.

Approach

1. Correlate the presence and dosage of the bioelectric modulators with wound healing.
2. Develop a collagen-collagen biocomposite with tailorable nanostructure and collagen fibril alignment, and study the relationship between collagen fiber topography and wound healing to enable optimization of the biocomposite structure.
3. Incorporate bioelectric modulators into the collagen-collagen composite and characterize release.
4. Measure endogenous EF at corneal wound sites and correlate with effects of bioelectric modulators, pharmacological delivery system, and collagen biocomposite to assess efficacy of these materials and pharmaceuticals for healing.



A collagen biocomposite will accelerate ocular wound healing via multiple wound healing strategies: biochemical cues, topographical cues and delivery of bioelectric modulators that enhance the wound EF.

Timeline and Cost

Aims	FY15	FY16	FY17
Correlation of bioelectric modulators to wound EF, healing			
Development of collagen biocomposite			
Encapsulation and release of bioelectric modulators			
Optimization of materials to maximize therapeutic benefit			
Cost	$316	$345	$339

Updated: 06 February 2014

Goals/Milestones

FY15 Goals – Selection of bioelectric modulators. Collagen biocomposite synthesis.
- ❑ Screening of pharmacological agents using rat corneal model (RCM) and human cornea test platform (HCTP)
- ❑ Alignment of collagen fibrils, fabrication of high strength gels

FY16 Goal – Correlation of topography effects on EF and wound healing. Engineering of tailored release of bioelectric modulators.
- ❑ Analysis using vibrating probe technique (RCM, HCTP)
- ❑ Characterization of release of agents from nanoparticles/fibers

FY17 Goal – Demonstration of efficacy of biomaterial-pharmacological agent combination for EF stimulation and wound healing.
- ❑ Optimization of collagen biocomposite topography and bioelectric agent delivery
- ❑ Quantification of effects on EF and healing (RCM, HCTP)

9. Appendices

None.